PHYTOCHEMICAL STUDY OF THE ARIAL PARTS OF *Cleome rutidosperma* DC PLANT

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Received 20 July 2008; Accepted 13 October 2008

ABSTRACT

Two new compounds were identified as 2-ethyl-cyclohex-2-ene-6-hydroxy-methylene-1-carboxylic acid and 3β-hydroxy-lup-20(29)-en-28-oic acid, respectively, from the petroleum ether extracts of Cleome rutidosperma plant. These two constituents is the first time occurrence in this plant. The structures of the two different type of compounds are elucidated with the help of UV, IR, ^{1}H-NMR, ^{13}C-NMR, COSY, DEPT 90, DEPT 135 and mass spectral data.

Keywords: *Cleome rutidosperm DC; isolation; spectral analysis*

INTRODUCTION

Cleome rutidosperma, locally known as 'Beguni hurhure' is a low growing herb, up to 70 cm tall, found in waste grounds and grassy places with trifoliate leaves and small violet-blue flowers, which turn pink as they age. The elongated capsules display the asymmetrical, dull black seeds. The plant is native to West Africa, from Gulnea to Nigeria, Zaire and Angola.

It has become naturalized in various parts of tropical America as well as Southeast Asia including the 'Indo-Bangla' sub-continent [1-3]. Ayurvedi, Yunani doctors and Local Kabiraj used the different parts like leaves, roots and seeds as stimulant, antiscorbutic, anthelminthic, rubifacient, vesicant and carminative [4]. The antiplasmodial, analgesic, lacomotor, antimicrobial, diuretic, laxative and anthelmintic activity of the certain extracts of the plant has already been reported [5-9]. Presence of phytoconstituents like terpenoids, saponins, flavonoids have been found to be responsible for diuretic and laxative activities of the ethanolic extract and its fractions of the plant [8]. Extensive chemical examination of the plant was carried out and several groups of compounds isolated such as terpenoids, saponins, flavonoids, alkaloids etc. In this paper, we describe the isolation and structure elucidation of the substituted cyclohexene and terpenoid type of compounds from two different fractions of petroleum ether extracts. To the best of our knowledge these two compounds has not been previously isolated or reported from this plant.

EXPERIMENTAL

Plant Material

The plant materials (without root) of *Cleome rutidosperma* were collected from the Curzon Hall Campus of the University of Dhaka, Bangladesh. The plant was identified and voucher specimen number was deposited at the Department of Botany, Dhaka University, Dhaka, Bangladesh with No. 041.

Spectroscopy

Melting point was determined on an electrochemical micro-melting point apparatus (Gallenkamp). The UV, IR (KBr) spectra were recorded on a Shimadzu UV-168A and Shimadzu IR-470A spectrophotometer, respectively. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker R-32 (400 MHz) in deuterated methanol (CD_3OD) with TMS as an internal standard (chemical shifts in δ, ppm). TLC was performed with silica gel GF_{254}. All solvents were analytical reagent grade. Mass spectra were recorded on a Varian Saturn 3800 GC-MS/MS spectrophotometer.

Extraction and Isolation

The arial parts of *Cleome rutidosperma* were washed with water to removed mud and all other dusty materials. The washed plant materials were dried at room temperature followed by in a control electrical oven at 40 °C. The dried plant materials were chopped into small pieces followed by grinding through a cyclotec grinder (200 mesh). These powdered materials were used in the present investigation. The powdered materials (800 g) was refluxed with petroleum ether (40-60 °C) for 72 hr in a Soxhlet extractor. After filtration the extract was concentrated under reduced pressure at 40 °C in a rotatory evaporator. The concentrated extract was dried by vacuum pump to yield dry mass. The dry mass was mixed with small amount of silica gel (60-120 mesh) maintaining the ratio (2:1) and dried in air. After drying the mixture was powdered in a mortar and applied to vacuum liquid chromatography (VLC) over TLC grade

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silica gel (GF_{254}). The column was initially eluted with petroleum ether (40-60 °C) followed by gradient elution with the mixture of petroleum ether with increasing amount of dichloromethane, 100% dichloromethane followed by the mixture of dichloromethane with increasing quantities of methanol and finally with methanol. These elutes were collected in a series of test tubes (more than 170 tubes) with 20 mL in each fraction. All these fractions were monitored by TLC (over silica gel GF_{254}). The elutes of similar behaviour (similar R_f values) were combined together to afforded fourteen fractions F_1 (3-7), F_2 (8-11), F_3 (12-15), F_4 (16-20), F_5 (21-32), F_6 (33-50), F_7 (51-59), F_8 (60-84), F_9 (85-112), F_{10} (113-125), F_{11} (130-142), F_{12} (143-155), F_{13} (156-169) and F_{14} (rest of the tubes). All these fractions were concentrated separately and allowed to stand at room temperature for a couple of weeks. A yellowish semi solid amorphous substance (4.8 mg) settled out from fraction F_4 and a white crystalline solid (5.3 mg) deposited from fraction F_9 and these two fractions were marked as CR_1 and CR_2, respectively.

Characterization of compounds CR_1 and CR_2

Compound CR_1: Yellowish semi solid amorphous (4.8 mg) was obtained from the fraction F_4. It could not be crystallized from any solvent. It was only soluble in hexane and chloroform solvents. R_f 0.60 (n-hexane-ethyl acetate; 9:2); (M^+, 184); UV: 228 nm; IR: 3400, 2900, 2850, 1735, 1575, 1486, 1450, 1370, 1150, 720 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR . Table-1.

***Compound CR_2*:** Obtained from column was further purified by preparative TLC over silica gel GF_{254} using n-hexane-ethyl acetate (9:2) as a developing solvent. It was recrystallized from hexane to give a white crystalline solid (5.3 mg); m.p. 297°C; R_f 0.56 (n-hexane-ethyl acetate; 9:2); (M^+, 458); UV: 276 nm; IR: 2900-2700, 1700, 1640-1600, 1475, 1365 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR. Table-2 redundancy

RESULT AND DISCUSSION

The compounds, 2-Ethyl-cyclohex-2-ene-6-hydroxy-methylene-1-carboxylic acid (**1**) and 3β-hydroxy-lup-20 (29)-en-28-oic acid (**2**) were isolated from this plant for the first time. Compound **1** was obtained as a yellowish amorphous solid. High-resolution mass of **1** indicated the molecular formula $C_{10}H_{16}O_3$ (M^+, 184). Its IR spectrum showed an absorption peak at 3400 cm^{-1} indicating the presence hydroxyl group (-OH) and the absorption bands at 2900-2850 cm^{-1} indicating the presence of –CH aliphatic asymmetric stretching. The absorption band at 1735 and 1575 cm^{-1} indicating the presence of ester carbonyl group (>C=O) and conjugated double bond, respectively. Two sharp absorption bands at 1450 and 1370 cm^{-1} indicating the presence of -CH_2 and -CH_3 groups, respectively. Two broad absorption peaks at 1140 and 720 cm^{-1} indicating the presence of -C-O stretching for secondary alcoholic group and -C-H stretching for >CH_2 groups present in the ring. The ^{1}H-NMR spectrum showed the chemical shift at δ 5.3 indicating the presence of one olefinic proton at position C-3 (**Table 1)**. A pair of doublet of doublet at δ 4.1 and 4.2 with the coupling constant *J*=6.12 indicating the presence of hydroxymethylene protons (H_A, H_B) at C-6 and the other doublet at δ 8.7 indicating the presence of one proton in –COOH group. Up field chemical shifts a multiplet at δ 2.02-2.20 indicated the presence of 2H protons (>CH_2) at C-4 and C-1, respectively. The down field chemical shift a broad singlet at δ 5.2 indicated the presence of one proton (–OH) of hydroxymethylene group. A broad singlet at δ 1.54-1.67 indicating the presence of four protons at H-1, H-5 and H-6. A triplet at δ 2.11 indicating of three methyl protons at C-1′. In the ^{13}C-NMR spectra of the compound **CR_1** revealed the presence of 10 carbons.

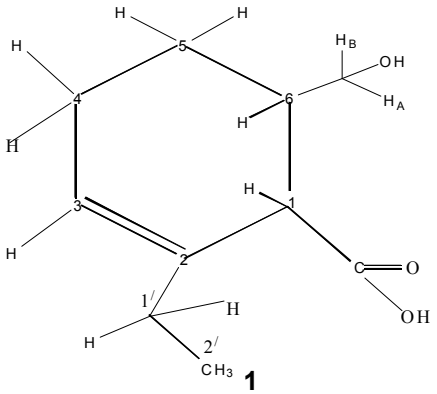

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Table 1. ^{1}H-NMR and ^{13}C-NMR Spectral data for compound CR_1 (2-ethyl-cyclohex-2-ene-6-hydroxymethylene-1-carboxylic acid (1)

Position	δ_H	δ_C	Position	δ_H	δ_C
1	1.54-1.67, m	68.90	1′	2.11, t	29.36
2	1.54-1.67, m	129.71	2′	2.13, s	14.12
3	5.3, m	130.25	3′	-	62.11
4	2.02, m	29.72	-COOH	8.7, d	173.31
5	1.54-1.67, m	29.54	-OH	5.2 , s	
6	4.2, dd, *J*=6.12	34.20			
	4.12, dd, *J*=6.12				

***** Assignments are based on DEPT 90 and DEPT 135, ^{1}H-NMR, ^{13}C-NMR, ^{1}H-COSY

The chemical shift at δ 68.9 indicating the presence of C-H carbon at position C-1 bearing the –COOH group. Three different chemical shifts at δ 29.36, 29.54 and 29.72 indicated the presence of methylene carbon attached at position C-1$^{/}$, C-5 and C-4, respectively. The chemical shift at δ 34.2 indicated the presence of C-H carbon at position C-6 bearing the -CH_2OH group. Two relatively down field chemical shifts at δ 129.7 and 130.25 inducting the presence of >C=C< between C-2 and C-3. The downfield chemical shift at δ 173.31 indicating the presence of carbonyl carbon of -COOH group and the up field chemical shift at δ 12.11 indicating the presence of oxymethylene carbon at C-3$^{/}$ linked through position C-6. The other up field signal at δ 14.12 indicating presence of methyl carbon at C-2$^{/}$ linked through C-1$^{/}$.

On the basis UV, IR, ^{1}H-NMR, ^{13}C-NMR, Dept 90, Dept 135 and mass spectral data and the other physical properties the isolated pure compound **CR_1** was identified and established as 2-ethyl-cyclohex-2-ene-6-hydroxymethylene-1-carboxylic acid **(1).**

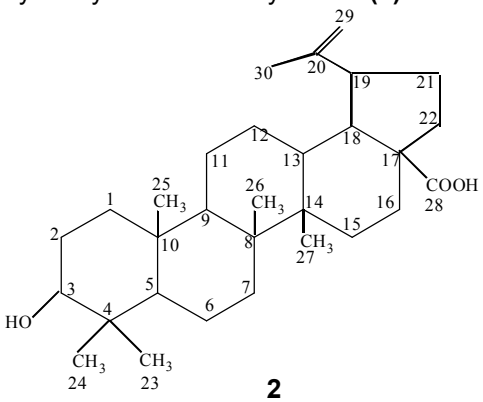

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Table 2. ^{1}H-NMR and ^{13}C-NMR Spectral data for compound CR_2 (3β-hydroxy-lup-20 (29)-en-28-oic acid (2).

Position	δ_H	δ_C	Position	δ_H	δ_C
1	2.22 , dd	39.3	17		61.2
2	1.2, m	28.3	18	1.80, m	49.5
3	2.22, dd	78.1	19	3.52, m	48.6
4		39.5	20		150.5
5	0.83, m	55.8	21		32.0
6	1.26, m	19.0	22	1.80, m	36.2
7		35.2	23	0.91, s	28.5
8		41.4	24	1.00, s	16.3
9	1.28, t	51.0	25	0.81, s	16.4
10		37.8	26	1.15, s	16.4
11	1.18, m	21.5	27	1.24, s	16.2
12	1.15, m	26.6	28		177.2
13	2.46, m	38.6	29	4.48, s	110.5
14		43.9	30	1.77, s	19.4
15	1.80, m	40.3	-COOH		173.21
16	4.09, d	75.1	-OH	5.2, s	

* Assignments are based on DEPT 90 and DEPT 135, ^{1}H-NMR, ^{13}C-NMR, ^{1}H-COSY

The compound **2** was obtained as white crystalline solid. It was highly soluble in hexane and chloroform successively. High-resolution mass spectra exhibited molecular ion at m/z 458, which is consistent with the molecular formula $C_{30}H_{48}O_3$ and confirmed by ^{1}H-NMR, ^{13}C-NMR (Table 2) and DEPT analysis.

The UV spectra displayed the characteristic absorption bands at 228 nm so there is no conjugated double bond in the structure. Its IR spectrum showed an absorption peak in the region 2900-2700 cm^{-1} indicating the presence of –CH aliphatic asymmetric stretching in conjugation and the absorption in peaks at 1700 and 1640 to 1600 cm^{-1} indicating the presence of carboxyl carbonyl group and asymmetric ethylenic double bond, respectively. The absorption bands at 1475 and 1365 cm^{-1} indicating the presence of -CH_3 and -CH_3 groups, respectively.

The ^{1}H-NMR spectrum showed the up field chemical shifts at δ 0.79, 0.81, 1.15 and 1.24 was due to the tertiary methyl groups of the triterpenoidal nature of the compounds. The chemical shift at δ 2.22 was the doublet of doublet with H-1 and H-3 intensity having the coupling constant *J*=2.18 and 11.0 was assigned for the double bond proton along with the three other methyl groups at that show the up field chemical shift at δ 0.83, 0.91 and 1.00 indicating the isopropenyl residue of the skeleton. Other protons have their usual chemical shift values. The ^{13}C-NMR spectra of the fraction revealed thirty carbon signals, which were assigned by Dept as seven methyl, ten methylene (including =CH_2), six methine, seven quaternary, one alcoholic methine and one carboxylic acid. In comparison with the data of oleanolic acid, the carbon signals for C-14, C-15, C-16, C-17 and C-18 appeared slightly or largely shifted (41.00→43.9, 35.20 →40.3, 32.8 →75.1, 56.6 →61.2, 47.7 →49.5). Therefore, on the basis of chemical shift the structure of the compound **CR_2** was determined to be 3β-Hydroxy-lup-20 (29)-en-28-oic acid (2).

ACKNOWLEDGEMENT

The authors are grateful to Mr. Aminul Ahsan, Senior Scientific Officer, Analytical Chemistry Division, BCSIR Laboratories, Bangladesh for her help in connection with DEPT, ^{1}H-NMR, ^{13}C-NMR, ^{1}H-COSY. They are also grateful to Dr. S. M. Salehuddin, Chemistry Division, Atomic Energy Centre, Ramna, Dhaka, Bangladesh for his help in connection with GC-MS/MS.

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